

STUDIES ON THE INFLUENCE OF HYDRAZINE, SUB-
STITUTED HYDRAZINES, AND RELATED
NITROGENOUS COMPOUNDS ON THE
CARBOHYDRATE METABOLISM
IN THE RABBIT

BY

SEIICHI IZUME

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY OF MICHIGAN

REPRINTED FROM

THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS,
VOL. XXX, No. 1, PAGES 87-93, NOVEMBER, 1926

AND FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. LXXI, No. 1, PAGES 33-66,
DECEMBER, 1926

1926

Am Arbor
1926



STUDIES ON THE INFLUENCE OF HYDRAZINE, SUB-
STITUTED HYDRAZINES, AND RELATED
NITROGENOUS COMPOUNDS ON THE
CARBOHYDRATE METABOLISM
IN THE RABBIT

By
SEIICHI IZUME

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY OF MICHIGAN

REPRINTED FROM

THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS,
VOL. XXX, No. 1, PAGES 87-93, NOVEMBER, 1926

AND FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. LXXI, No. 1, PAGES 33-66,
DECEMBER, 1926

1926



Digitized by the Internet Archive
in 2026

THE INFLUENCE OF HYDRAZINE AND ITS DERIVATIVES ON METABOLISM.

II. CHANGES IN THE NON-PROTEIN NITROGENOUS CONSTITU- ENTS OF THE BLOOD AND IN THE METABOLISM OF INJECTED GLYCINE IN HYDRAZINE INTOXICATION.

BY HOWARD B. LEWIS AND SEIICHI IZUME.

*(From the Laboratory of Physiological Chemistry, Medical School, University
of Michigan, Ann Arbor.)*

(Received for publication, September 30, 1926.)

INTRODUCTION.

Mann and his coworkers (1-4) have recently reviewed the rôle of the liver in many important metabolic processes and, with the aid of the improved operative technique for hepatectomy developed by him, have brought forward new and convincing evidence of the function of the liver in the regulation of blood sugar content, in the deamination of amino acids, in the formation of urea, and in the destruction of uric acid.

There is another method available by which these hepatic functions may be demonstrated; namely, by the use of chemical substances which have an effect primarily upon the liver cells without producing marked injury to other tissues. Among these substances is hydrazine, which has been shown by Wells (5) to act specifically upon the parenchymatous cells of the liver. Although many investigations have been concerned with the influence of hydrazine on carbohydrate metabolism, little attention has apparently been given to the question of protein or uric acid metabolism in the hydrazinized animal. It is the purpose of the present paper to present data obtained on the influence of

hydrazine poisoning in the rabbit upon the amino acid, urea, and uric acid content of the blood, and upon the glyconeogenesis following ingestion of a simple amino acid, glycine.

It has been observed by Underhill (6), MacAdam (7), and Bodansky (8) that the subcutaneous injection of hydrazine sulfate produces a notable hypoglycemia. Underhill (9) has also reported that the total solids of the blood are increased in hydrazine poisoning, an increase associated with the anhydremia produced (10, 11), which has been ascribed to the fluid loss through vomiting. Underhill and Baumann (12) have demonstrated a hyperlipemia in hydrazinized dogs, the maximum content of blood fat being definitely related to the hypoglycemia. Hendrix and McAmis (13) observed an alkalosis in hydrazine poisoning, but were unable to show any causal relation between the alkalosis and the hypoglycemia. The ratio of sodium to chlorine in the serum was found to be increased in hydrazine intoxication. Bodansky (14) has reported one experiment in which he noted a marked increase in the non-protein nitrogen of the blood of a dog poisoned with hydrazine. Underhill and Kleiner (15) found that the urinary nitrogen and sulfur were little altered in dogs after the administration of hydrazine, but the content of creatinine and phosphorus was somewhat increased. No lactic, hydroxybutyric, or diacetic acid, no acetone and no glucose or other reducing substances were present in the urine. MacAdam (7) observed a creatinuria in hydrazine intoxication, which was closely associated with the hypoglycemia. These results were confirmed by Underhill and Baumann (16) who also noted a decreased hydrogen ion concentration of the urine.

In addition to the histological demonstration of liver injury in hydrazine poisoning (5), functional tests have also indicated impairment of the activity of the hepatic cells. The rate of excretion of injected phenoltetrachlorophthalein (17) is greatly decreased in dogs after hydrazine administration. The ability to synthesize hippuric acid after the administration of sodium benzoate is diminished (18). The tolerance for fructose is also lessened (14). Fiske and Karsner (19), however, in perfusion experiments, were not able to demonstrate any difference between normal livers and livers poisoned with hydrazine in their ability to lower the ammonia content of the perfusion fluid.

EXPERIMENTAL.

Methods.

Rabbits which had fasted for several days were used in this investigation. Blood was usually drawn from the marginal ear vein, but occasionally from the heart, sodium citrate being used as anticoagulant. Both hydrazine sulfate and glycine were dissolved in physiological salt solution and injected subcutaneously. The animals received no food during the course of the experiments, but were given 50 to 75 cc. of water twice daily through a stomach tube. It was hoped by this means to avoid any marked concentration of the blood, which might be associated with the fasting and hydrazine intoxication.

For the determination of sugar, non-protein nitrogen, urea nitrogen, and amino nitrogen of the blood, and amino nitrogen of the urine, the methods of Folin and Folin and Wu were used. Uric acid in the blood was estimated directly in the Folin-Wu tungstic acid filtrate by Benedict's method. The glycogen content of the liver was determined by Pflüger's method with a slight modification in the technique of the final determination of reducing sugar. After the conversion of glycogen into glucose in the usual way, the solution was decolorized with Lloyd's reagent and the glucose was then determined by the Folin-Wu method.

*Results.**Influence of Hydrazine on Amino Acid, Urea, and Uric Acid Content of Blood.*

Our experiments judged both from the chemical findings and from the results of pathological examination of the liver and kidneys fall into two rather clearly defined groups. Microscopic examination¹ of the tissues by Dr. A. S. Warthin of the Department of Pathology showed that a sufficient dose of hydrazine sulfate (130 mg. per kilo) when administered subcutaneously to rabbits always produced to some extent a fatty degenerative infiltration of the liver. This finding was constant in practically all of our animals. In about half of these, however, the kidneys

¹ The pathological examination and diagnosis were made by Dr. A. S. Warthin, to whom we wish to take this opportunity to express our thanks for his hearty cooperation.

36 Hydrazine Influence on Metabolism. II

were also affected, inflammation and fatty degenerative infiltration being observed.

In those animals in which kidney involvement resulted following

TABLE I.
Rabbit 109, female, weight 1.9 kilos.

Date.	Hydrazine sulfate injected.	Blood content of:					Remarks.
		Sugar.	Non-protein nitrogen.	Urea nitrogen.	Amino acid nitrogen.	Total color with uric acid reagent calculated as uric acid.	
1926	mg. per kg.	mg.	mg.	mg.	mg.	mg.	
June 8							6 days fasting.
9.00 a.m.							
10.50 "	130	109	60.7	26.5	10.3	1.80	
4.50 p.m.		163	66.7	30.1	16.7	2.23	
10.50 "		122	74.5	37.0	18.4		
June 9							
10.50 a.m.		110	105.3	55.2	17.6		
10.50 p.m.		150	148.2	84.8	17.5	2.76	Slight depression.
June 10							
10.50 a.m.		125	165.8	114.3	20.3	2.89	
11.30 "	130						
1.30 p.m.		74	210.0	124.0	30.4	4.36	Marked depression. Death.
2.00 "							

Pathological Report.—Kidney showed very marked lesions. Throughout the cortex there were areas of atrophy and fibrosis with some inflammatory infiltration involving a whole labyrinth. The tubules were in part atrophic and in part dilated with atrophy of the cells, but the glomeruli were relatively well preserved in these areas. In some of the dilated tubules a finely granular bluish-staining precipitate was present. Some of the tubules showed a slight lipoidosis of renal cells. The inflammatory process extended clear through from the cortex to the portion of the papilla belonging to the part involved.

Liver: slight fatty degenerative infiltration. Atrophy of large bile ducts. Slight lymphocyte infiltration of some of the periportal islands.

the injection of hydrazine, a marked rise in the non-protein nitrogen and in the urea nitrogen occurred (Table I). It was noted that, whenever the blood showed a marked retention of

urea nitrogen, the kidney injury was also present. We consider that the rise in the urea content of the blood is associated with a disturbance of kidney function and that the hepatic injury is not necessarily involved.

TABLE II.

Rabbit 113, female, weight 2.4 kilos.

Date.	Hydrazine sulfate injected. <i>mg.</i> <i>per kg.</i>	Blood content of:					Remarks.
		Sugar.	Non-protein nitrogen.	Urea nitrogen.	Amino acid nitrogen.	Total color with uric acid reagent calculated as uric acid.	
		<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	
1926							
July 2							7 days fasting.
9.40 a.m.		95	60.6	32.5	10.3	2.29	
10.15 "	130						
1.15 p.m.		87	62.0	33.3	12.1	2.86	
4.15 "		62	61.7	25.8	15.8	2.47	
7.15 "		105	72.3	26.0	22.4	2.38	
10.15 "		154	84.5	30.2	26.0	2.38	
July 3							
10.15 a.m.		111	102.5	32.8	32.2	2.96	Depression, blood from heart.
July 4							
10.15 a.m.		111	86.9	33.8	31.1	3.86	Still in depression.
July 5							
10.15 p.m.		106	70.2	33.6	13.5	2.96	Apparently normal. Killed.

Pathological Report.—Postmortem microscopical examination showed: (1) Liver: Moderate passive congestion. Slight small celled infiltration of many islands. Slight hepatitis. Slight atrophy of liver cells without fatty infiltration but scattered cells showed large fat droplets (slight fatty infiltration). No necrosis. (2) Kidney: Very slight cloudy swelling, possibly post mortem. No other pathology.

In the absence of kidney lesions, the content of non-protein nitrogen, amino acid nitrogen, and uric acid always increased prominently and progressively following the injection of hydrazine sulfate, while the urea nitrogen remained almost constant (Tables II and III). Of the changes in the composition of the blood, the

38 Hydrazine Influence on Metabolism. II

increase in the amino acid nitrogen was most significant, its maximum content being two to four times the normal value for the fasting rabbit. The increase cannot be considered to be due to any concentration of the blood, since the urea nitrogen was practically unchanged and the sugar content frequently much lowered. No definite relationship between the degree of hypoglycemia and the increase of amino acid nitrogen was noted, but

TABLE III.
Rabbit 106, female, weight 2.3 kilos.

Date.	Hydrazine sulfate injected.	Blood content of:					Remarks.
		Sugar.	Non-protein nitrogen.	Urea nitrogen.	Amino acid nitrogen.	Total color with uric acid reagent calculated as uric acid.	
<i>1926</i>	<i>mg. per kg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	
June 2 4.20 p.m.	100	107	51.5	24.5	14.1	1.27	6 days fasting.
5.15 "							
June 3 12.15 a.m.	100	132	55.3	24.2	19.5	1.31	Coma. Condition continued. Death.
8.15 "		142	61.4	25.8	24.7	1.50	
10.05 "							
4.05 p.m.		42	72.1	24.7	31.8	3.66	
6.05 "		39	78.4	23.5	34.3	3.70	
7.00 "							

Pathological Report.—(1) Liver: Very slight fatty degenerative infiltration. Congestion. (2) Kidney: Congestion. Slight cloudy swelling. No other changes found.

it was observed that the progressive increase in the amino acid nitrogen was usually paralleled by the increase in uric acid.² The increased amino acid content was most marked when the animals

² We use the term "uric acid" content with the realization that the substance determined by Benedict's direct method, particularly in rabbit blood, may consist in large part of other substances which react with the arsenophosphotungstic acid reagent.

showed severe intoxication. Thus Rabbit 113 developed the maximum increase (213 per cent of the normal content) 24 hours after the administration of hydrazine when the animal showed severe symptoms of intoxication. During the intoxication, the blood amino acid content continued high, but when the animal recovered from the symptoms and appeared normal 84 hours after the injection, the amino acid content was also found to have returned nearly to the normal value. It should be noted that the greater part of the increase in the non-protein nitrogen is accounted for by the rise in the amino acid nitrogen. The results of these and other similar experiments suggest that in hydrazine

TABLE IV.
Effect of Subcutaneous Injection of Glycine upon Amino Acid Nitrogen Content of Blood of Normal Fasting Rabbits.

Rabbit No.	Fasting.	Glycine injected per kilo.	Amino acid nitrogen in 100 cc. blood.					
			Initial.	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
	<i>days</i>	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
99a	2	1.0	8.23	17.5	17.5	14.0		
101a	2	1.0	8.14	18.5	17.5	14.3		
102	5	1.0	9.95		26.2	21.4	13.4	
103	5	1.0	8.58		22.5	18.4	12.3	
108	6	1.0	8.91	24.2	23.1	16.8	10.3	9.5
99b	4	3.0*	8.23	49.0	47.2	47.0		40.0
101b	4	3.0*	8.14	46.4	48.2	45.3		43.8

* Previous injection of glycine (1 gm. per kilo) on the 2nd day of fasting.

poisoning, the injury to the liver results in a disturbance of protein metabolism as evidenced by the failure of deamination of amino acids and their accumulation in the blood.

Influence of Hydrazine on Amino Acid Tolerance of Rabbits and on Glyconeogenesis Following Administration of Glycine.

Further evidence of the failure of the animal organism to metabolize the protein derivatives satisfactorily in hydrazine poisoning was afforded by comparative studies of the behavior of glycine injected subcutaneously into normal and hydrazinized rabbits. As shown in Table IV, when glycine in moderate amounts (1 gm. per kilo) was injected into rabbits previously fasted for short periods,

the amino acid nitrogen content of the blood increased to two to three times the initial value within an hour, and remained high for several hours, gradually returning to the normal content within 9 to 12 hours. With larger amounts of glycine (3 gm. per kilo) the increase was much greater and the high level of blood amino acid nitrogen persisted for more than 12 hours.

When glycine (1 gm. per kilo) was injected after previous injec-

TABLE V.

Effect of Subcutaneous Injection of Glycine upon Amino Acid Nitrogen Content of Blood under Influence of Hydrazine Poisoning.

Rabbit No.	Fasting.	Admin- istration of:		Amino acid nitrogen in 100 cc. blood.										Remarks.
				Initial.	After hydrazine.		After glycine.					mg.		
					3 hrs.	6 hrs.	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.			
days	mg.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.			
102	6	130	1.0	9.7	12.4	17.5		37.0	40.3	42.0		Glycine given 7 hrs. after hydrazine.		
103	6	130	1.0	11.0	12.9	14.9		38.5	47.3	52.5		Glycine given 6.5 hrs. after hydrazine.		
108	7	130	1.0	8.5	12.5	15.5	35.6	40.2	46.0	49.2	49.0	Glycine given 7 hrs. after hydrazine.		
99	6	130	3.0	8.2	15.5*		63.7	86.5	95.5			Glycine given 5 hrs. after hydrazine.		
101	6	130	3.0	8.1	16.6*		73.3	82.5	86.5			" "		

* Sample was collected 4 hours after the injection of hydrazine.

tion of hydrazine (Table V), the amino acid content of the blood, which was already somewhat increased as a result of the hydrazine injection, increased to a maximum of three to four times the initial value, and remained at this high level for more than 12 hours. With larger amounts of glycine (3 gm. per kilo), the blood amino acid had increased tenfold in 6 hours, when the animals were killed for the determination of glycogen in the liver.

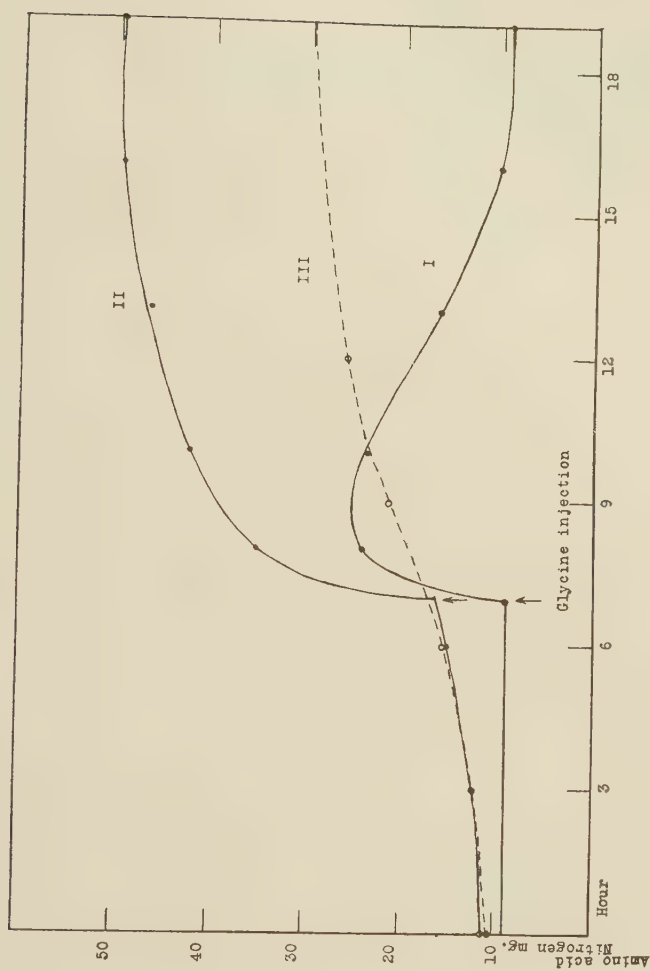


FIG. 1. A comparison of the amino acid nitrogen content of the blood following the injection of 1 gm. of glycine per kilo (a) in a fasting rabbit (No. 108) (Curve I) and (b) in the same rabbit after hydrazine poisoning (Curve II). Curve III represents the changes in amino acid nitrogen of the blood in a fasting rabbit (No. 113) which had been injected with hydrazine but had received no glycine.

42 Hydrazine Influence on Metabolism. II

A graphic representation of this difference between normal and hydrazinized animals is given in Fig. 1, in which are shown the curves for the amino acid nitrogen of the blood following the

TABLE VI.

Effect of Subcutaneous Injection of Glycine upon Urea Nitrogen Content of Blood in Inanition and after Administration of Hydrazine.

Rabbit 108, male, weight 1.9 kilos.

Date.	Hydrazine sulfate injected.	Glycine injected.	Blood content of:		
			Sugar.	Amino acid nitrogen.	Urea nitrogen.
1928	mg. per kg.	gm. per kg.	mg.	mg.	mg.
June 1*					
8.45 a.m.			109	8.9	26.2
8.55 "		1			
9.55 "			125	24.2	27.4
11.55 "			111	23.1	20.7
2.55 p.m.			105	16.8	34.5
5.55 "			100	10.3	32.2
8.55 "			111	9.5	30.3
June 2					
8.10 a.m.			102	8.5	27.6
8.50 "	130				
11.50 "			75	12.5	26.1
2.50 p.m.			56	15.5	23.4
3.20 "		1			
4.20 "			98	35.6	23.2
6.20 "			95	40.2	25.5
9.20 "			82	46.0	25.0
June 3					
12.20 a.m.			126	49.2	27.1
3.20 "			135	49.0	25.1
11.50 "			100	32.0	26.8
11.50 p.m.			105	31.5	26.5
June 5					
10.00 a.m.			112	9.8	27.5

* The animal fasted for 6 days previous to the beginning of the experiment.

injection of 1 gm. of glycine per kilo in the fasting rabbit (Curve I), and in the same animal under the influence of hydrazine intoxication (Curve II). For comparison, the changes in the blood amino acid content of another rabbit poisoned with hydrazine, but to

which no glycine was administered, are given (Curve III). It is evident that the ability of the organism to metabolize injected glycine is greatly impaired in hydrazine poisoning.

In the normal fasting rabbit the urea content of the blood after the injection of glycine (1 gm. per kilo) increased gradually and reached its maximum value about 6 hours after the injection. In the hydrazinized rabbit, on the other hand, even though the amino acid nitrogen of the blood increased greatly above the normal after the injection of glycine, no rise in the urea nitrogen was observed. A typical protocol is presented in Table VI in which are given the values for blood urea of Rabbit 108, the same animal for which

TABLE VII.

Effect of Subcutaneous Injection of Glycine upon Sugar Content of Blood.

Rabbit No.	Fasting.	Glycine injected per kilo.	Blood sugar content in 100 cc. blood.					
			Initial.	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
99a	2	1.0	0.118	0.132	0.117	0.112		
101a	2	1.0	0.116	0.120	0.111	0.107		
102	5	1.0	0.124		0.129	0.121	0.105	
103	5	1.0	0.134		0.123	0.116	0.111	
108	6	1.0	0.109	0.125	0.111	0.105	0.100	0.111
100a	2	1.0	0.124	0.131	0.128	0.118		
99b	4	3.0*	0.118	0.154	0.155	0.148		0.143
101b	4	3.0*	0.116	0.143	0.143	0.143		0.141
100b	4	3.0*	0.124	0.150	0.141	0.133		0.123

* Previous injection of glycine (1 gm. per kilo) on the 2nd day.

curves of blood amino acid content are plotted in Fig. 1. The constancy of the values for urea nitrogen obtained in this experiment would indicate that the high values for amino acid nitrogen cannot be due to concentration of the blood as a result of the hydrazine intoxication.

The blood sugar of fasting rabbits was little affected by the injection of glycine in doses of 1 gm. per kilo (Table VII). In most animals the sugar content of the blood remained unaffected, but occasionally (*e.g.* Rabbits 99a and 108, Table VII) a slight transient hyperglycemia was observed 1 hour after the administration of glycine. However, when larger doses of glycine (3

44 Hydrazine Influence on Metabolism. II

gm. per kilo) were injected, the blood sugar content increased about 20 to 30 per cent in an hour and remained high for several hours. In the hydrazinized animals, even when 3 gm. of glycine per kilo were injected, no significant change in the blood sugar was

TABLE VIII.
Effect of Subcutaneous Injection of Glycine upon Sugar Content of Blood under Influence of Hydrazine Poisoning.

Rabbit No.	Fasting. days	Admini- stration of:		Sugar content of 100 cc. blood.								Remarks.
		Hydrazine sul- fate per kilo.		Initial. gm.	After hydrazine.			After glycine.				
		mg.	gm.		3 hrs. gm.	4 hrs. gm.	6 hrs. gm.	1 hr. gm.	3 hrs. gm.	6 hrs. gm.	9 hrs. gm.	
102	6	130	1.0	0.133	0.145		0.132		0.138	0.133	0.158	Glycine given 7 hrs. after hydra- zine.
103	6	130	1.0	0.134	0.159		0.131		0.111	0.108	0.100	Glycine given 6.5 hrs. after hydra- zine.
108	7	130	1.0	0.102	0.075		0.056	0.098	0.095	0.082	0.126	Glycine given 7 hrs. after hydra- zine.
99	6	130	3.0	0.118		0.153		0.146	0.143	0.160		Glycine given 5 hrs. after hydra- zine.
101	6	130	3.0	0.116		0.111		0.116	0.120	0.166		" "

apparent (Table VIII). Thus the blood sugar of Rabbit 99 which had increased from 0.118 to 0.154 per cent 1 hour after the glycine injection (Table VII) showed no change after glycine when the animal had been previously poisoned with hydrazine (Table

VIII). It is evident that the transformation of glycine to glucose does not proceed normally under the influence of hydrazine intoxication.

TABLE IX.

Glycogen Content of Liver Following Subcutaneous Injection of Glycine to Normal and Hydrazinized Rabbits.

Rabbit No.	Body weight.	Previous of period of fasting.	Injection.		Glycogen content of liver.	Remarks.
			Hydrazine sulfate.	Glycine.		
	kg.	days	mg. per kg.	gm. per kg.	per cent	
111	1.6	7	0	0	0.0927	Control animal.
8	1.2	4	100	0	0.136	Died 1 hr. after hydrazine injection.
109	1.9	8	130 130	0	0.047	Died 2.5 hrs. after last dose of hydrazine. Hydrazine given on 6th and 8th days.
7	2.8	7	100 100 100	0	0.080	Died 4 hrs. after last dose of hydrazine. Hydrazine given on 4th, 6th, and 7th days.
106	2.3	7	100 100	0	0.058	Died 6 hrs. after last dose of hydrazine. Hydrazine given on 6th and 7th days.
102	2.0	6	130	1.0	0.147	Killed 9 hrs. after glycine injection. Hydrazine given 7 hrs. before glycine.
103	2.3	6	130	1.0	0.087	" "
104	2.4	7	0	3.0	0.061	Killed 9 hrs. after glycine injection.
105	2.9	6	0	3.0	0.624	" "
112	1.7	7	0	3.0	0.762	" "
99	2.6	6	130	3.0	0.218	Killed 7 hrs. after glycine injection. Hydrazine given 5 hrs. before glycine.
101	2.0	6	130	3.0	0.126	" "

A study of the deposition of glycogen after glycine injection was made in hydrazine poisoning (Table IX). In accord with the work of previous investigators we have observed that very small quantities of glycogen are present after fasting and after fasting

and hydrazine intoxication (Rabbits 7, 8, 106, 109, 111). The contents of glycogen in the liver of two rabbits (Rabbits 99 and 101), which after 6 days fasting were injected with hydrazine sulfate and 5 hours later with glycine (3 gm. per kilo), were found to be 0.218 and 0.126 per cent. Control rabbits similarly treated but which received no hydrazine (Rabbits 104, 105, and 112) stored 0.061, 0.624, and 0.762 per cent glycogen in the liver, indicating in two of the three animals the formation of appreciable amounts of glycogen after glycine injection. It appears that both glucose and glycogen formation from glycine are inhibited as a result of the injury to the liver produced by hydrazine.

DISCUSSION.

The marked increase in the amino acid content of the blood following the administration of hydrazine, might be considered to be due to an increased catabolism of tissue proteins, to a decreased ability of the organism to deaminize and utilize amino acids, or to a difficulty in excreting amino acids through the kidneys. Underhill and Kleiner (15) observed that the excretion of total nitrogen and sulfur in animals during hydrazine poisoning was only slightly different from that obtained during a comparable period of inanition. This fact seems to indicate that hydrazine probably does not induce an increased catabolism of tissue proteins to any considerable extent. The fact, which has been demonstrated by the authors, that the increase in amino acids in the blood in hydrazine poisoning was not always accompanied by an increase in blood urea, indicates that the accumulation of amino acids in the blood cannot always be ascribed to the renal injury which has been shown to be produced sometimes in rabbits by hydrazine.

It would seem reasonable, therefore, to attribute the increase of the amino acid content of the blood to injury of the liver which is known to occur in hydrazine poisoning, through which the power to deaminize and metabolize amino acids is disturbed. There is considerable evidence that the liver is an organ importantly concerned with the deamination of amino acids and the formation of urea. Severe injuries to or extirpation of the liver might be expected to cause an accumulation of amino acids in the blood and a decrease in the urea content of the blood and urine. Bollman, Mann, and Magath (3) studied the effect of complete hepatectomy

on the formation of urea in dogs and noted that the amino acid content of the blood increased progressively while the blood urea decreased considerably. A high amino acid content of the blood has also been observed in hepatic disease, acute yellow atrophy, cirrhosis, tumor, etc. (20-24). These facts lead us to believe that the increased amino acid content of the blood after the injection of hydrazine are due to the derangement of hepatic function produced by the poison.

The changes of amino acid and urea content of the blood observed in fasting rabbits after the injection of glycine in our experiments are in accord with those obtained by Van Slyke and Meyer (25) and Seth and Luck (26). They are also confirmed by unpublished experiments of Johnston in this laboratory. Van Slyke and Meyer also found that after the intravenous injection of 0.853 gm. of glycine per kilo into a dog, only 3.5 per cent of the injected glycine was recovered in the urine. In our fasting rabbits, the increases in the amino acid nitrogen in the urine after the subcutaneous administration of 1 gm. of glycine per kilo are comparable to that of Van Slyke, 1.2 to 6.7 per cent of injected glycine being excreted in the urine.

The decreased ability of the hydrazinized rabbit to deaminate and metabolize injected glycine, which we attribute to liver injury, is also in harmony with other experimental evidence. Rosenbaum (21) introduced a mixture of amino acids, "rectamin," in doses equivalent to 40 to 60 mg. of amino nitrogen per kilo subcutaneously into normal individuals and into patients with hepatic diseases (tumor, cirrhosis, yellow atrophy, etc.). In normal individuals the change in the amino acid nitrogen content of the blood was not significant, while in cases of hepatic disease a marked hyperaminoacidemia was always observed within a short time after the injection. Similar results are reported by von Falkenhausen (24), Glaessner (27), Masuda (28), and Bier (29).

SUMMARY.

1. Subcutaneous administration of hydrazine sulfate to fasting rabbits often produced injury to the kidneys, as well as fatty degeneration of the liver.

2. Hydrazine always caused a marked rise in the amino acid content of the blood. The urea content of the blood following the

injection of hydrazine to rabbits was not increased when the kidneys were not affected by the hydrazine, but often increased markedly when the kidneys showed lesions.

3. In hydrazine poisoning the power of the animal organism to metabolize injected glycine, to transform glycine into glucose and urea, and to synthesize glycogen from glycine, was diminished.

4. The results are ascribed to the derangement of the function of the liver which results in the failure of normal deamination of amino acids. These results also furnish evidence that the liver is importantly concerned with deamination of amino acids and formation of urea.

Addendum.—After this paper was submitted for publication, further evidences of the similarity between the results observed in hydrazine poisoning and total hepatectomy were reported by Bollman, Mann, and Magath (30). In hepatectomized dogs it was demonstrated that no deaminization of injected amino acids occurred, and no formation of urea could be observed. It was also seen that no glucose was formed from amino acids (glycine, alanine) after the removal of the liver. Results obtained in dogs after hepatectomy by these observers are quite comparable to the results we have obtained after amino acid injection in hydrazinized rabbits.

BIBLIOGRAPHY.

1. Mann, F. C., and Magath, T. B., *Arch. Int. Med.*, 1922, xxx, 73.
2. Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1921, lv, 285.
3. Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1924, lxix, 371.
4. Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1925, lxxii, 629.
5. Wells, H. G., *J. Exp. Med.*, 1908, x, 457.
6. Underhill, F. P., *J. Biol. Chem.*, 1911-12, x, 159.
7. MacAdam, W., *Biochem. J.*, 1915, ix, 229.
8. Bodansky, M., *Am. J. Physiol.*, 1923, lxvi, 375.
9. Underhill, F. P., *J. Biol. Chem.*, 1914, xvii, 293.
10. Underhill, F. P., and Karelitz, S., Jr., *J. Biol. Chem.*, 1923-24, lviii, 147.
11. Bodansky, M., *J. Pharmacol. and Exp. Therap.*, 1924, xxiii, 127.
12. Underhill, F. P., and Baumann, E. J., *J. Biol. Chem.*, 1916, xxvii, 169.
13. Hendrix, B. M., and McAmis, A. J., *J. Biol. Chem.*, 1924, lxi, 45.
14. Bodansky, M., *J. Biol. Chem.*, 1923-24, lviii, 799.
15. Underhill, F. P., and Kleiner, I. S., *J. Biol. Chem.*, 1908, iv, 165.
16. Underhill, F. P., and Baumann, E. J., *J. Biol. Chem.*, 1916, xxvii, 151.
17. Whipple, G. H., Mason, V. R., and Peightal, T. C., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 207.

18. Lackner, E., Levinson, A., and Morse, W., *Biochem. J.*, 1918, xii, 184.
19. Fiske, C. H., and Karsner, H. T., *J. Biol. Chem.*, 1914, xviii, 381.
20. Chesney, A. M., Marshall, E. K., and Rowntree, L. G., *J. Am. Med. Assn.*, 1914, lxiii, 1533.
21. Rosenbaum, S., *Z. ges. exp. Med.*, 1924, xli, 420.
22. Stadie, W. C., and Van Slyke, D. D., *Arch. Int. Med.*, 1920, xxv, 693.
23. Schweringer, F., *Z. exp. Path. u. Therap.*, 1920, xxi, 129.
24. von Falkenhausen, M. F., *Arch. exp. Path. u. Pharmacol.*, 1924, ciii, 322.
25. Van Slyke, D. D., and Meyer, G. M., *J. Biol. Chem.*, 1913-14, xvi, 213.
26. Seth, T. N., and Luck, J. M., *Biochem. J.*, 1925, xix, 366.
27. Glaessner, K., *Z. exp. Path. u. Therap.*, 1907, iv, 336.
28. Masuda, N., *Z. exp. Path. u. Therap.*, 1911, viii, 629.
29. Bier, J., *Wien. klin. Rundschau*, 1913, xxvii, 529.
30. Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1926, lxxviii, 258.

THE INFLUENCE OF HYDRAZINE AND ITS DERIVATIVES ON METABOLISM.

III. THE MECHANISM OF HYDRAZINE HYPOGLYCEMIA.

By SEIICHI IZUME AND HOWARD B. LEWIS.

*(From the Laboratory of Physiological Chemistry, Medical School,
University of Michigan, Ann Arbor.)*

(Received for publication, September 30, 1926.)

INTRODUCTION.

The mechanism of the hypoglycemia produced by the injection of hydrazine sulfate has been studied from various points of view by Underhill and his coworkers. They finally concluded from their studies on the respiratory metabolism that the hypoglycemia was probably due to an increased combustion of carbohydrate. Without enumerating the facts which indicate that the hypoglycemia and other physiological effects produced by hydrazine are quite different from those produced by the internal secretion of the pancreas, insulin, it is evident that the effect of hydrazine on carbohydrate metabolism cannot be explained satisfactorily by the hypothesis of Underhill.

In a previous paper of this series (1) the authors have reported that the administration of hydrazine to fasting rabbits caused the derangement of liver function, which was manifested in an increase in the amino acid content of the blood and in a decrease in the ability to metabolize injected glycine, to transform glycine to glucose and urea, and to synthesize glycogen in the liver from glycine. In the present paper, experimental evidence is presented which indicates that the hypoglycemia following the injection of hydrazine is also closely associated with hepatic injury, which results in the failure of glycogenesis in the transformation of amino acids to glucose. On basis of these observations a theory of the mechanism of hydrazine hypoglycemia is suggested which differs somewhat from that of Underhill.

Since Underhill's discovery of the production of hypoglycemia by hydrazine (2), the mechanism of the hypoglycemia has been studied by various methods in his laboratory. Underhill and Fine (3) were able to prevent hyperglycemia and glycosuria in depancreatized dogs by the injection of hydrazine, and were able to demonstrate the presence of epinephrine in the adrenals of hydrazinized dogs. These results led them to the conclusion that hydrazine probably induced hypoglycemia by increasing the efficiency of the pancreatic secretion or by augmenting its output, since no inhibitory influence on the adrenal secretion was evidenced. The glycogen content of the liver and muscles of dogs was greatly decreased following the administration of hydrazine (4). Underhill and Prince (5) were unable to demonstrate any differences in the degree of utilization of glucose in the perfusion of the surviving hearts of normal and hydrazinized rabbits. Rabbits poisoned with hydrazine showed a marked retardation in the utilization of glucose introduced subcutaneously (6). The glyoxalase activity of the liver was not altered by hydrazine (7). Underhill and Murlin (8) observed that the administration of hydrazine to fasting dogs caused an increased respiratory quotient. The heat production of these animals was not increased, however, to any extent, by the hydrazine. They concluded that an increased combustion of carbohydrate, evidenced by the greater respiratory quotient, was the probable cause of the hypoglycemia.

If, in hydrazine intoxication, there is sufficiently great increase in carbohydrate combustion to account for the hypoglycemia observed, then it would be anticipated that the glucose administered would be more rapidly utilized by the animal under the influence of hydrazine than by the normal animal. Underhill and Hogan (6), however, were unable to show that hydrazine led to a more rapid utilization of glucose by the organism, but, on the contrary, found that the hyperglycemia following the subcutaneous injection of glucose persisted longer in hydrazinized animals than in normal animals. The authors have repeated the experiments of Underhill and Hogan and were not only able to confirm their results, but have offered further evidence which indicates that this decrease in the tolerance for glucose is to be

ascribed to an impairment of the glycogenetic function of the liver produced by hydrazine.

Underhill has noted a difference in response to injected glucose between dogs and rabbits in hydrazine intoxication. Hydrazinized dogs were killed by the subcutaneous injection of 5 gm. of glucose per kilo, while rabbits under the same conditions showed marked and persistent hyperglycemia, but survived the injections. Bodansky (9) who studied the glucose tolerance of dogs in hydrazine poisoning does not appear to have observed the death of his animals after glucose injection as did Underhill.

EXPERIMENTAL.

In these experiments, slightly larger doses of hydrazine sulfate and smaller amounts of glucose than those employed by the former investigators were used in order to produce more effective hydrazine intoxication and to prevent, as far as possible, the excretion of glucose into the urine. The rabbits received no food throughout the course of the experiments, but 50 to 75 cc. of water were given through a stomach tube twice daily. After a preliminary period of fasting, usually of 2 days duration, rabbits were injected subcutaneously with hydrazine sulfate in doses of 130 mg. per kilo. Several hours after, when the animals usually showed abnormal symptoms, 1.85 gm. of glucose (in 25 per cent solution) per kilo were injected subcutaneously. The sugar content of the blood was determined by the Folin-Wu procedure as indicated in the tables. 6 to 8 hours after the injection of glucose the animals were killed and the glycogen content of the liver was determined by a slight modification of Pflüger's method (1). The control animals received no hydrazine but were treated similarly in other respects.

The data presented in Table I indicate that, after the injection of glucose (1.85 gm. per kilo) to fasting rabbits, the concentration of blood sugar was increased by about 50 per cent at its maximum and usually returned to the normal level within 3 hours. They also demonstrate a considerable storage of glycogen in the liver 6 hours after the administration of glucose.

When, however, the same dosage of glucose was injected into fasting rabbits which had been previously poisoned with hydra-

TABLE I.
Glucose Tolerance of Normal Rabbits.

Rabbit No.	Weight.	Fast-ing.	Injection of glucose per kilo.	Blood sugar content.								Liver glyco-gen.
				Initial.	After injection of glucose.							
					0.5 hr.	1 hr.	1.5 hrs.	2 hrs.	3 hrs.	6 hrs.		
	kg.	days	gm.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
80	3.25	1	1.85	0.130	0.202	0.200	0.181	0.145	0.129			
82	2.1	3	1.85	0.129	0.207	0.193	0.193	0.174	0.134	0.120		
83	2.2	3	1.85	0.139	0.175	0.169	0.135	0.129	0.109	0.112		
84	3.1	1	1.85	0.087	0.103	0.104	0.101	0.104	0.104			
85	3.2	1	1.85	0.114	0.164	0.187	0.150	0.138	0.123			
86	3.1	2	1.85	0.128		0.246			0.150	0.133	1.68	
87	3.6	2	1.85	0.128		0.185			0.133	0.138	5.65	
88	2.9	1	1.85	0.123			0.151		0.118			
89	4.1	1	1.85	0.118			0.154		0.125			
90	2.4	2	1.85	0.132		0.192		0.164	0.134	0.122	2.30	

TABLE II.
*Effect of Administration of Hydrazine Sulfate (130 Mg. per Kilo) upon
Glucose Tolerance of Rabbits.*

Rabbit No.	Weight.	Fasting.	Blood sugar content.								Liver glyco-gen.	Remarks.
			Initial.	After hydra-zine.	After injection of glucose (1.85 gm. per kilo).							
					1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	8 hrs.		
	kg.	days	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
78	2.7	1	0.134	0.153			0.367			0.241		Convul-sions, stopped by glu-cose in-jection. " "
80	3.2	1	0.130	0.087	0.200		0.190		0.196		0.187	
84	3.0	2	0.087	0.093	0.153		0.154		0.121			
85	3.2	2	0.114	0.060	0.103	0.143		0.168	0.222	0.222	0.109	
88	2.9	2	0.123	0.105		0.174		0.176		0.150	0.183	
89	4.1	2	0.118	0.084		0.210		0.214		0.230	0.181	

zine, the hyperglycemia became more marked (Table II), with an average maximum increase of 90 per cent, and persisted for more than 6 hours as a rule. The glycogen deposited in the liver after glucose injection was very slight in the hydrazinized animal, the average content of the liver glycogen of four rabbits being 0.165 per cent. In both series of experiments the loss of glucose by excretion through the kidneys was found to be almost negligible, the maximum excretion being less than 10 mg. per hour. The difference in the hyperglycemia following the injection of glucose

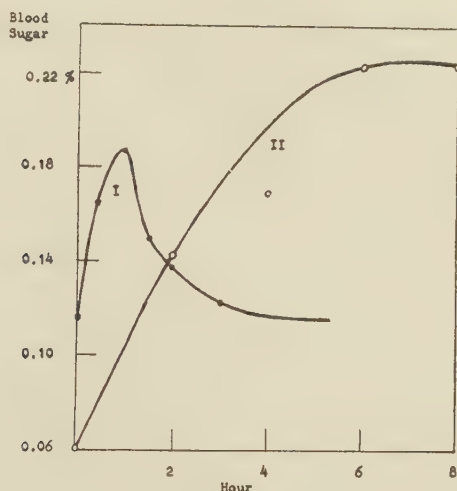


FIG. 1. A comparison of the glucose tolerance as shown by the variations in the glucose content of the blood following the subcutaneous injection of 1.85 gm. of glucose per kilo in the fasting animal (Rabbit 85) (Curve I) and in the same animal after hydrazine poisoning (Curve II).

in normal fasting rabbits and in hydrazinized rabbits is shown graphically in Fig. 1 in which the tolerance test for glucose is shown for Rabbit 85 before (Curve I) and after (Curve II) the administration of hydrazine.

Our observations concerning the hyperglycemia in normal and hydrazinized rabbits after the injection of glucose support the work of Underhill and Hogan (6) previously mentioned. The values for the glycogen of the liver are also in accord with those of Sato (10). 3 hours after the intravenous injection of 2.5 gm. of

glucose per kilo, the liver of rabbits which had undergone a preliminary period of inanition contained about 2 per cent of glycogen, while in control animals, similarly treated, but which received no glucose, the glycogen content was approximately 0.45 per cent.

Although it had previously been observed (6,9) that the glucose tolerance was diminished in hydrazine intoxication, no studies of the ability of the liver to form glycogen after glucose administration in hydrazine poisoning appear to have been made. We have demonstrated that, despite the persistent hyperglycemia after glucose administration in hydrazinized animals, the liver was unable to transform the excess glucose into glycogen. Hydrazine apparently so injures the hepatic cells that the glycogenetic function of the liver is deranged and this injury is manifested by the decrease in the glucose tolerance. This point of view is supported by the fact that a definite decrease in the tolerance for glucose is reported in hepatic injury, as in yellow atrophy produced by phosphorus poisoning (11). Clinical observations also lend support to this argument (12-17).

Since the power to synthesize glucose to glycogen was shown to be affected by hydrazine, it seemed of interest to determine whether the transformation of some simple carbon compound, normally a glucose former, to glucose was influenced under the conditions of hydrazine intoxication. The influence of hydrazine intoxication on the metabolic changes of lactic acid, which is closely related physiologically to glucose and also a probable intermediary product of the metabolism of alanine, was investigated.

That lactic acid may be converted to glucose in both depancreatized (18) and phlorhizinized (19) dogs has been long known and frequently confirmed (20-23). Röhmann (24) demonstrated that the administration of sodium or ammonium lactate to fasting rabbits led to glycogen storage in the liver. Perfusion experiments have also shown the possibility of glycogen formation from lactic acid in the turtle liver (20). Underhill and Blatherwick (25), who noted that the hypoglycemia following thyreoparathyroidectomy was relieved by the injection of calcium lactate, considered this effect to be due to the calcium ion rather than to lactic acid. Parnas and Wagner (26), who reported an interesting

case of hypoglycemia associated with a tumor of the liver in a young girl, observed that the oral administration of calcium lactate was effective in restoring the blood sugar temporarily to its normal level, and considered that the effect was due to the conversion of lactic acid to glucose. Pollak (27) did not observe any rise in blood sugar content after the subcutaneous injection of 1.3 gm. of lithium lactate into a rabbit of 2.2 kilos. This negative result is probably to be ascribed to the relatively small amount of lactate injected.

TABLE III.

Effect of Administration of Sodium Lactate on Sugar Content of Blood and on Glycogen Content of Liver.

Rabbit No.	Weight.	Fasting.	Sodium lactate as lactic acid per kilo.	Blood sugar content.					Liver glycogen.	Remarks.
				Initial.	After administration of sodium lactate.					
					1 hr.	3 hrs.	6 hrs.	9 hrs.		
	kg.	days	gm.	per cent	per cent	per cent	per cent	per cent		
90	2.6	2	5.0	0.143	0.211	0.231	0.174	0.154		Administration
91	2.6	2	5.0	0.114	0.240	0.297	0.214*			per os.
92	3.1	1	3.0	0.133	0.152	0.182		0.189*		" "
93a	2.8	2	3.0	0.144	0.157	0.191	0.200	0.108		" "
93b	2.7	3	3.0	0.114	0.150	0.186	0.178	0.123	1.85	Subcutaneous administration.
94	2.8	2	3.0	0.103	0.144	0.174	0.138	0.113		" "
95	2.8	4	3.0	0.116	0.135	0.158	0.167	0.129	2.09	" "
96	2.1	2	3.0	0.125	0.146	0.154	0.133	0.120		" "
97	2.2	2	3.0	0.101	0.120	0.118	0.121	0.110		" "

* Dyspnea, death.

The general plan of the present series of experiments was identical with that of the glucose series already presented. The lactic acid (inactive) was injected as the sodium salt, in 10 or 25 per cent solution, in doses usually equivalent to 3 gm. of lactic acid per kilo of body weight. As a rule the injection of this amount of sodium lactate resulted in the production of a hyperglycemia (Table III), the content of blood sugar reaching its maximum

58 Hydrazine Influence on Metabolism. III

(approximately 43 per cent higher than the normal figure) in about 3 hours and returning to the normal level within 9 hours. No abnormal symptoms were observed. A similar hyperglycemia followed the oral administration of the same amount of sodium lactate to two animals. One of these, however, showed marked depression and died 20 hours after the administration. Oral administration of larger doses (5 gm. per kilo) induced a more marked hyperglycemia, but was fatal in one of the two experiments, the rabbit dying in $6\frac{1}{2}$ hours. In confirmation of the work of others, storage of glycogen was observed in the two animals killed for the glycogen determination (1.85 and 2.09 per cent of glycogen in the liver, Table III).

When sodium lactate was injected 6 hours after the previous injection of hydrazine, the magnitude and duration of the resultant

TABLE IV.
Effect of Hydrazine upon Hyperglycemia and Storage of Glycogen in Liver Following Subcutaneous Injection of Sodium Lactate.

Rabbit No.	Weight.	Fasting.	Hydrazine sulfate per kilo.	Sodium lac- tate as lactic acid per kilo.	Blood sugar content.							Liver glycogen.
					Initial.	After hy- dra- zine.	After sodium lactate.					
							1 hr.	3 hrs.	6 hrs.	9 hrs.		
<i>kg.</i>	<i>days</i>	<i>mg.</i>	<i>gm.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
94	2.8	3	130	3.0	0.103	0.154	0.151	0.200	0.185	0.177	0.092	
96	2.1	3	130	3.0	0.125	0.115	0.145	0.181	0.185	0.139	0.022	
97	2.2	3	130	3.0	0.101	0.084	0.110	0.176	0.143	0.143	0.760	

hyperglycemia (Table IV) were greater than those observed in normal fasting rabbits, the average maximum increase of the blood sugar being 72 per cent and the duration of the hyperglycemia more than 9 hours. The glycogen content of the liver 10 hours after the injection of the lactate was low, especially in two of the three animals studied (Table IV).

The blood sugar curves following the injection of sodium lactate before and after the administration of hydrazine for Rabbit 96 are shown in Fig. 2 and need no further comment.

The storage of glycogen in the liver of normal fasting rabbits which we observed is comparable to that obtained by Röhmann (24). He fed rabbits which had previously fasted for 3 to 4 days

a few doses of 2.5 gm. of sodium lactate together with 2.5 gm. of sodium acetate (in order to prevent the oxidation of lactic acid) at intervals of a few hours and killed the animals 3 to 21 hours after the last administration. The livers of the animals which were fed the lactate contained about 1.86 per cent of glycogen, while control experiments in which no lactate was administered showed an almost complete lack of glycogen.

We have been unable to find any reference in the literature to the production of hyperglycemia in normal animals following the

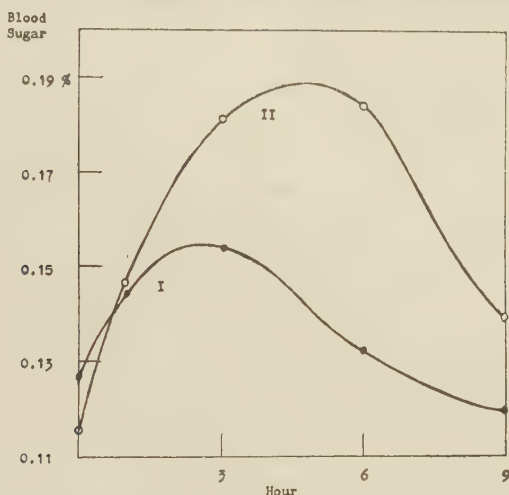


FIG. 2. The influence of hydrazine on the development of the hyperglycemia following the injection of sodium lactate (equivalent to 3 gm. of lactic acid per kilo) in Rabbit 96. Curve I shows the blood sugar values in the fasting period, and Curve II the values in hydrazine intoxication.

administration of lactate. Pollak's failure to observe any significant change after the subcutaneous injection of lithium lactate (27) may be due to the small amount injected, since the difference in the cations, *i.e.* lithium and sodium, can hardly explain the difference between his results and ours. Pollak injected lithium lactate in an amount equivalent to 0.55 gm. of lactic acid per kilo. We have observed that, as a rule, sodium lactate in doses less than 2.0 gm. as lactic acid per kilo did not induce any appreciable hyperglycemia.

The possibility was suggested that some oxidation product of lactic acid, *e.g.* pyruvic acid, with the power of reducing alkaline copper solutions might be formed in the organism and be estimated as glucose in the blood sugar determination. Blood after the administration of sodium lactate was tested for the presence of pyruvic acid by the method of Hurlley (28). It was not possible to detect pyruvic acid in any of the blood examined, although, in control tests, it was noted that positive tests could be obtained at a dilution of 1 part of pyruvic acid in 50,000 parts of the tungstic acid blood filtrate. These results make it improbable that the hyperglycemia observed was due to pyruvic acid.

These results obtained in the demonstration of the production of hyperglycemia and the formation of glycogen in the liver after the administration of sodium lactate to the normal animal furnish further evidence that lactic acid is a precursor of glucose in the organism.

Under like conditions a similar hyperglycemia was observed in hydrazinized animals. This indicates that hydrazine does not interfere, at least to any great extent, with the ability of the organism to transform lactic acid to glucose. The greater degree of hyperglycemia and its continuation over a longer period of time, observed in hydrazinized animals, indicate that, as previously shown, the ability to transform glucose to glycogen is greatly diminished by hydrazine. These conclusions are also supported by the experimental results of Underhill and Hogan (7). They observed that the glyoxalase activity of the liver of dogs was not altered appreciably by hydrazine. According to Dakin and Dudley (29), the conversion of lactic acid to glucose may be effected with the aid of the enzyme, glyoxalase, methyl glyoxal being formed as an intermediary product in this transformation.



The activity of the glyoxalase in the liver and other tissues may be considered, therefore, as one of the indexes of the capacity of the animal organism to transform lactic acid to glucose.

It is characteristic of the hypoglycemias produced by insulin and by hepatectomy that convulsions result when an extremely low level of blood sugar is reached. These convulsions may be relieved and the animals restored to normal conditions for several

hours by the administration of glucose (30). In our studies of hydrazine poisoning, six of nine rabbits which received hydrazine sulfate in doses of 130 mg. per kilo developed convulsions. Immediately after the onset of the convulsions was observed, a 25 per cent solution of glucose (1.85 gm. per kilo) was injected subcutaneously. In four animals the administration of glucose produced no effect upon the convulsions and the animals died within a short time, while in the other two animals the convulsions ceased promptly after the injection of glucose. The animals were not restored, however, to the normal condition, but were usually depressed and finally became comatose and remained so, in one case, for more than 30 hours before death occurred. It is probable that, although the symptoms which are closely associated with hypoglycemia were relieved, death may have been due to the injury of functions of the liver other than those associated with carbohydrate storage, or to the injury of other organs produced by hydrazine. Williamson and Mann (31) observed that two dogs in a series of twenty-six, which received chloroform, developed convulsions which were relieved in one animal by glucose. Wierzechowski (32) observed convulsions in three of ten dogs under the influence of phlorhizin and was able to relieve the convulsions within 20 minutes by the oral administration of glucose.

DISCUSSION.

On the Mechanism of the Hypoglycemia Produced by Hydrazine.

In general, hypoglycemia may be the result of two different types of reaction in the organism. (1) The decreased sugar content may be due to an increased rate of disappearance of sugar from the blood. This may be the result of (a) an increase in the combustion of glucose or glycolysis, (b) an increase in the rate of withdrawal of glucose for storage purposes or an increased rate of glycogenesis, or (c) an increased excretion of glucose or intermediary products of metabolism through the kidneys. (2) The lowered blood sugar may be the result of a decrease in the supply of sugar available in the system. Such a condition might arise either if glycogenolysis, the conversion of glycogen to glucose, is interfered with, or if glyconeogenesis, the conversion of non-carbohydrate materials to glucose, does not occur normally.

There is no evidence to show that the hydrazine poisoning induces an increased glycogenesis in the animal organism or that it facilitates the excretion of glucose or the intermediary products of carbohydrate metabolism through the kidneys. It was noted by Underhill (4) that after hydrazine injection the glycogen content of the liver and muscles markedly decreased and was never found to rise. Underhill and Kleiner (33) found no increased glucose or other reducing substances, and no lactic, hydroxybutyric, and diacetic acids or acetone bodies in the urine of hydrazinized dogs. We have not been able to demonstrate any increased excretion of glucose in hydrazinized animals following glucose administration, even when a marked hyperglycemia persisted as a result of glucose injections.

There is, however, evidence which has been interpreted as indicating an increased glycolysis after hydrazine administration (8). The respiratory quotient of fasting dogs increased slightly following the administration of hydrazine.¹ But this cannot be interpreted to be the result of an increased rate of glycolysis alone, rather than of changes in the character of the metabolic processes, because the following facts are not in accord with this assumption. (1) The basal metabolism of the dogs was not increased by the hydrazine (8). (2) The consumption of glucose by the surviving heart of the rabbit was not increased by the administration of hydrazine (5). (3) The utilization of the glucose which was parenterally introduced into the hydrazinized animal was never greater than that observed in the normal animal (6)

¹ Underhill and Murlin (8) state that "subcutaneously introduced dextrose is oxidized more rapidly in dogs that have received hydrazine than in the normal fasting animal." In support of this statement one fasting control experiment alone is cited (Dog 4). It should be noted that the conditions were not comparable, as the fasting animal, although heavier than the other animals, received the same amount of glucose. The fasting animal also fasted only 2 days while the hydrazinized dogs had fasted 13 days (10 days prior to hydrazine and 3 days after its injection) and 7 days (5 days prior to the injection of hydrazine and 2 days after). In a later experiment when this animal was injected with hydrazine and the rate of combustion of subcutaneously introduced glucose studied, the weight of glucose injected was double that previously injected for the fasting control. It is difficult to interpret these data because of this lack of adequate control experiments.

if the degree and duration of the hyperglycemia be made the criterion.

It would seem that the increased respiratory quotient, which was observed by Underhill and Murlin (8) in hydrazinized animals, was a manifestation of the hepatic injury caused by the poison. Porges (34) and Porges and Salomon (35) found that a rise in the respiratory quotient followed ligation of the abdominal aorta and the inferior vena cava below the diaphragm both in normal rabbits and in depancreatized dogs. Similar results were obtained by Rolly (36), Murlin, Edelmann, and Kramer (37), and others. Grafe and Denecke (38) and Mann (39) also observed that the respiratory quotient was usually raised after the total extirpation of the liver in dogs. Porges considered that the fact was the result of increased carbohydrate combustion, fat and protein being more difficultly oxidized in the absence of the liver; while Rolly and Murlin and his coworkers, who demonstrated that there was always a fall in the carbon dioxide content of the blood whenever the respiratory quotient of the operated animal was increased, considered that the increase was the result of pulmonary overventilation caused mechanically by the effect of the operation and not the result of an alteration in the character of the metabolism as thought by Porges. In hydrazine poisoning, as shown by Hendrix and McAmis (40), the pH and carbon dioxide combining-power of the blood is always increased, and, therefore, the increase in the respiratory quotient of the animal after the administration cannot be considered solely as the result of the pulmonary overventilation.

An explanation of the mechanism of the hydrazine hypoglycemia must, therefore, be sought in the second type of reaction; *i.e.*, the decrease in the supply of glucose available for combustion. There is no evidence that glycogenolysis is diminished, since the glycogen content of the liver after glucose injection is less in hydrazinized animals than in normal fasting animals. There are, however, reasons to believe that the protein metabolism is deranged and that the normal formation of sugar from protein (amino acids), the most important type of glycconeogenesis, is diminished quantitatively in hydrazine intoxication (1). Moreover, the occurrence of lipemia in hydrazine poisoning (41) indicates a derangement in the normal metabolism of fat.

The series of reactions which are suggested to occur in hydrazine poisoning is somewhat as follows. Hydrazine injures the hepatic cells and induces conditions under which the transformation of non-carbohydrate materials into glucose and glycogen (glyconeogenesis), as well as the synthesis of glycogen from glucose and related substances, does not occur normally. Under these conditions the glycogen store of the liver, which is constantly depleted by glycogenolysis, cannot be maintained from the fat and protein stores of the organism and its amount is greatly diminished. A large amount of fat is mobilized from the storage depots and enters the liver cells, producing the lipemia and fatty infiltration of the liver commonly observed in hydrazine poisoning. As a result of hepatic injury, the normal deamination of amino acids does not occur and hyper amino acidemia results with the consequent failure of glyconeogenesis. Under these conditions, the fat and protein metabolism being disturbed, the organism utilizes chiefly carbohydrate, and this change in the character of the metabolism manifests itself in the increased respiratory quotient. The increase in the consumption of carbohydrate together with the limited supply of carbohydrate available as a result of diminished glyconeogenesis eventually produces the hypoglycemia characteristic of hydrazine poisoning.

SUMMARY.

1. Glycogen synthesis did not take place in the liver of hydrazinized rabbits, despite the marked hyperglycemia which followed the injection of glucose. This derangement of the glycogenetic function of the liver is considered to be the cause of the decreased glucose tolerance of rabbits poisoned with hydrazine.

2. The administration of sodium lactate to rabbits was found to induce a development of hyperglycemia and an accumulation of glycogen in the liver. The hyperglycemia produced by sodium lactate was intensified in its magnitude and duration by the previous injection of hydrazine, while the synthesis of the liver glycogen from sodium lactate was markedly diminished under these conditions.

3. Convulsions and other symptoms similar to those following hepatectomy were often observed in the rabbit during hydrazine

poisoning. In some cases the convulsions were immediately alleviated by the injection of glucose.

4. The mechanism of the hypoglycemia produced by hydrazine is discussed. It is suggested that the primary cause is failure of normal glyconeogenesis, the transformation of non-carbohydrate materials to glucose, as a result of which the supply of glucose available is diminished because of the hepatic injury produced by hydrazine.

BIBLIOGRAPHY.

1. Lewis, H. B., and Izume, S., *J. Biol. Chem.*, 1926, lxxi, 33.
2. Underhill, F. P., *J. Biol. Chem.*, 1911-12, x, 159.
3. Underhill, F. P., and Fine, M. S., *J. Biol. Chem.*, 1911-12, x, 271.
4. Underhill, F. P., *J. Biol. Chem.*, 1914, xvii, 293.
5. Underhill, F. P., and Prince, A. L., *J. Biol. Chem.*, 1914, xvii, 299.
6. Underhill, F. P., and Hogan, A. G., *J. Biol. Chem.*, 1915, xx, 203.
7. Underhill, F. P., and Hogan, A. G., *J. Biol. Chem.*, 1915, xx, 211.
8. Underhill, F. P., and Murlin, J. R., *J. Biol. Chem.*, 1915, xxii, 499.
9. Bodansky, M., *J. Biol. Chem.*, 1923-24, lviii, 799.
10. Sato, K., *Tohoku J. Exp. Med.*, 1923, iv, 312.
11. Frank, E., and Isaac, S., *Arch. exp. Path. u. Pharmacol.*, 1910, lxiv, 274.
12. Gilbert, A., and Baudouin, A., *Compt. rend. Soc. Biol.*, 1908, lxv, 710.
13. Tachau, H., *Deutsch. Arch. klin. Med.*, 1911, civ, 437.
14. Hetényi, G., *Deutsch. med. Woch.*, 1922, xlviii, 1200.
15. Pezzali, G., *Pathologica*, 1923, xv, 663, 690.
16. Albertoni, P., *Policlinico sez. med.*, 1922, xxix, 349.
17. Elek, L., and Goldgruber, G., *Z. ges. exp. Med.*, 1925, xlv, 130.
18. Embden, G., and Salomon, H., *Beitr. chem. Physiol. u. Path.*, 1904, v, 517; 1904, vi, 63.
19. Mandel, A. R., and Lusk, G., *Am. J. Physiol.*, 1906, xvi, 129.
20. Parnas, J., and Baer, J., *Biochem. Z.*, 1912, xli, 386.
21. Dakin, H. D., and Dudley, H. W., *J. Biol. Chem.*, 1913, xv, 127.
22. Puff, R., *Beitr. Physiol.*, 1922, ii, 7; *Chem. Abst.*, 1923, xvii, 2002.
23. Aubel, E., and Wursmer, R., *Compt. rend. Acad.*, 1923, clxxvii, 836.
24. Röhmman, F., *Arch. ges. Physiol.*, 1886, xxxix, 21.
25. Underhill, F. P., and Blatherwick, N. R., *J. Biol. Chem.*, 1914, xix, 119.
26. Parnas, J. K., and Wagner, R., *Biochem. Z.*, 1922, cxxvii, 55.
27. Pollak, L., *Biochem. Z.*, 1922, cxxvii, 120.
28. Hurtley, cited by Plimmer, R. H. A., *Practical organic and biochemistry*, London, 1915, 113.
29. Dakin, H. D., and Dudley, H. W., *J. Biol. Chem.*, 1913, xiv, 155, 423, 555; 1913, xv, 127, 463.
30. Mann, F. C., and Magath, T. B., *Arch. Int. Med.*, 1922, xxx, 171; *Am. J. Physiol.*, 1921, lv, 285.
31. Williamson, C. S., and Mann, F. C., *Am. J. Physiol.*, 1923, lxv, 267.

66 Hydrazine Influence on Metabolism. III

32. Wierzuchowski, M., *J. Biol. Chem.*, 1926, lxxvii, p. xlii.
33. Underhill, F. P., and Kleiner, I. S., *J. Biol. Chem.*, 1908, iv, 165.
34. Porges, O., *Biochem. Z.*, 1910, xxvii, 131.
35. Porges, O., and Salomon, H., *Biochem. Z.*, 1910, xxvii, 143.
36. Rolly, F., *Deutsch. Arch. klin. Med.*, 1912, cv, 494.
37. Murlin, J. R., Edelman, L., and Kramer, B., *J. Biol. Chem.*, 1913-14, xvi, 79.
38. Grafe, W., and Denecke, G., *Deutsch. Arch. klin. Med.*, 1915, cxviii, 249.
39. Mann, F. C., *Am. J. Physiol.*, 1923, lxiii, 397; *Ergebn. Physiol.*, 1924, xxiii, 212; and private communication.
40. Hendrix, B. M., and McAmis, A. J., *J. Biol. Chem.*, 1924, lxi, 45.
41. Underhill, F. P., and Baumann, E. J., *J. Biol. Chem.*, 1916, xxvii, 151.

THE INFLUENCE OF HYDRAZINE AND ITS DERIVATIVES ON METABOLISM

I. THE EFFECT OF SUBSTITUTION IN THE HYDRAZINE MOLECULE UPON THE HYPOGLYCEMIC ACTION OF HYDRAZINE¹

SEIICHI IZUME AND HOWARD B. LEWIS

From the Laboratory of Physiological Chemistry of the School of Medicine, University of Michigan, Ann Arbor

Received for publication September 20, 1926

INTRODUCTION

The discovery of insulin and of its characteristic effect upon the content of blood sugar has stimulated interest in other substances, which produce similarly hypoglycemia in the animal organism. Among these agents is hydrazine, whose hypoglycemic action was first noted by Underhill in 1911 (1). It has been the purpose of the authors in the series of papers of which this is the first to study the influence of hydrazine on carbohydrate metabolism, with especial reference to the mechanism of its action, since the current explanation of the hypoglycemia produced by hydrazine did not seem to be entirely adequate. In connection with this work, a series of derivatives of hydrazine has been prepared and their toxicity and effect upon blood sugar concentration in rabbits have been studied. It was hoped that, among these derivatives, there might be found some which produced the same hypoglycemia as hydrazine itself, but which, unlike hydrazine, were sufficiently non-toxic to make possible a more careful study of the mechanism of hydrazine hypoglycemia, and even to be of possible value therapeutically. While

¹ An abstract of a portion of the thesis presented by Seiichi Izume in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of the University of Michigan.

TABLE 2
The effect of some organic derivatives of hydrazine on the blood sugar concentration

SUBSTANCE	FORMULA	MINIMUM EFFECTIVE DOSE AS HYDRAZINE	EFFECTIVE IN THE PRODUCTION OF	
			Hypo- glycemia	Hyper- glycemia
1. Hydrazine hydrate.....	$\text{NH}_2 \cdot \text{NH}_2 \cdot \text{H}_2\text{O}$	mg. 32.0	+	-
2. Acetylhydrazine.....	$\text{CH}_3\text{CO} \cdot \text{NH} \cdot \text{NH}_2$	50.0	+	+
3. Benzoylhydrazine.....	$\text{C}_6\text{H}_5\text{CO} \cdot \text{NH} \cdot \text{NH}_2$	23.5	+	(?) *
4. Hippurylhydrazine.....	$\text{C}_6\text{H}_5\text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH} \cdot \text{NH}_2$	60.0	+	+
5. s-Diacetylhydrazine.....	$\text{CH}_3\text{CO} \cdot \text{NH} \cdot \text{NH} \cdot \text{COCH}_3$		+	+
6. s-Acetylbenzoylhydrazine.....	$\text{CH}_3\text{CO} \cdot \text{NH} \cdot \text{NH} \cdot \text{COCH}_2\text{C}_6\text{H}_5$	57.2	-	-
7. Ethylc hydrazinecarboxylate.....	$\text{C}_2\text{H}_5\text{CO}_2 \cdot \text{NH} \cdot \text{NH} \cdot \text{CO}_2\text{C}_2\text{H}_5$	40.0	-	-
8. Malonyldihydrazine.....	$\text{NH}_2 \cdot \text{NH} \cdot \text{CO} \cdot \text{CH}_2\text{CO} \cdot \text{NH} \cdot \text{NH}_2$		-	+
9. Bidimethylazimethylene.....	$(\text{CH}_3)_2\text{C} : \text{N} : \text{N} : \text{C} : (\text{CH}_3)_2$	30.0	+	-
10. Isopropylideneacetylhydrazine.....	$(\text{CH}_3)_2\text{C} : \text{N} \cdot \text{NH} \cdot \text{COCH}_3$	84.0	+	-
11. Isopropylidenebenzoylhydrazine.....	$(\text{CH}_3)_2\text{C} : \text{N} \cdot \text{NH} \cdot \text{COCH}_2\text{C}_6\text{H}_5$	18.2	+	-

* When both hypo- and hyperglycemia were observed, the hyperglycemia usually occurred first, following a period of excitement, while in the later stages of depression, hypoglycemia was observed. This hyperglycemia in the initial stage is frequently observed in poisoning with hydrazine itself, before the appearance of the hypoglycemia.

tically always fatal, the animals surviving this dosage in a few instances only.

A typical protocol of an experiment with hydrazine hydrate follows (table 1).

In table 2, are listed the organic derivatives of hydrazine studied with their formulae and characteristic actions.

The introduction of the acetyl, malonyl, ethylic carboxyl, and hippuryl radicals decreased the hypoglycemic effect while that of the benzoyl group tended to increase the effect slightly. The introduction of an acetyl group into monoacetylhydrazine and monobenzoylhydrazine greatly lessened the hypoglycemic action of these compounds. *s*-Dibenzoylhydrazine was prepared but proved to be too insoluble for injection in the required dosage. The introduction of the isopropylidene radical into hydrazine, acetylhydrazine and benzoylhydrazine did not markedly affect the hypoglycemia produced, all of the derivatives producing a hypoglycemia on subcutaneous injection.

All of these substances were toxic, but, in general, the introduction of the acetyl group decreased the toxicity. Thus for acetyl- and benzoylhydrazine, the lethal dosages per kilogram of body weight calculated as hydrazine were 50 and 24 mgm. respectively, while for the *s*-diacetyl- and *s*-acetylbenzoylhydrazine, the corresponding lethal dosages were greater than 140 and 57 mgm. (as hydrazine) respectively.

Since benzoylhydrazine was found to be equally as effective in the production of hypoglycemia as hydrazine, the influence of the introduction of a hydroxyl group into the benzene ring was studied with the three monohydroxybenzoylhydrazines. The *o*-derivative (2-hydroxybenzoylhydrazine) showed practically the same toxicity as benzoylhydrazine, but produced a marked hyperglycemia. The *m*-derivative (3-hydroxybenzoylhydrazine) was less toxic than benzoylhydrazine, the lethal dosage being more than three times as great, and produced no change in the concentration of blood sugar, while the *p*-derivate (4-hydroxybenzoylhydrazine) was similar in its degree of toxicity to the *m*-derivative. Its effect on blood sugar was not marked; in two animals, a slight hypoglycemia followed the injection.

The fact that the o-derivative is much more toxic than the m- and p-derivatives is in harmony with observations on other hydroxy-compounds, such as cresol, dihydroxybenzene, and hydroxybenzoic acid. Of the three isomeric cresols, the toxicity and cardiac depressant action was found by Gibbs and Hare (7) to be greatest in the case of o-cresol. It was also reported by the same observers (8) that 1,2-dihydroxybenzene (pyrocatechol) was more toxic than its isomers, the lethal dose of pyrocatechol being 0.04 to 0.05 grams per kilogram, of resorcinol, 0.7 to 1.0 grams, and of hydroquinone, 0.8 to 1.0 grams. 2-Hydroxybenzoic acid (salicylic acid) has long been known to possess powerful antiseptic and antipyretic properties, while the 3- and 4-hydroxybenzoic acids are inert physiologically (9).

Underhill (4) was unable to observe any production of hypoglycemia in a dog after the injection of semicarbazide hydrochloride ($\text{NH}_2 \cdot \text{NH} \cdot \text{CO} \cdot \text{NH}_2 \cdot \text{HCl}$). We have observed a marked hypoglycemia in the rabbit in one animal after the injection of 114 mgm. per kilogram (equivalent to 30 mgm. hydrazine). When, however, bivalent sulfur replaced oxygen as in thiosemicarbazide ($\text{NH}_2 \cdot \text{NH} \cdot \text{CS} \cdot \text{NH}_2$) the toxicity was greatly increased, 14 to 15 mgm. per kilogram (equivalent to 5 mgm. of hydrazine) producing a period of marked excitement, accompanied by a high blood sugar, which terminated fatally in a few hours. Similar toxicity was shown for another sulfur derivative, diammonium dithiocarbazate ($\text{NH}_2 \cdot \text{NH} \cdot \text{CS} \cdot \text{SH} \cdot \text{NH}_2 \cdot \text{NH}_2$). Doses of 17 to 44 mgm. per kilogram (equivalent to 5 to 20 mgm. of hydrazine) caused death in a few hours. No marked effect on the blood sugar concentration was noted. This increased toxicity due to the presence of sulfur has been observed in other organic groups, as, for example, the thiohydantoins and thio-piperazines (10).

SUMMARY

1. A series of hydrazine derivatives has been prepared and the influence of these on the concentration of blood sugar in the rabbit has been studied.

2. Hydrazine hydrate and its salts (sulfate, acetate) were

found to have nearly equal hypoglycemic effect and toxicity, when the content of hydrazine was comparable. The anion is not considered to influence the action of hydrazine.

3. The following derivatives of hydrazine were demonstrated to produce hypoglycemia in the rabbit: benzoylhydrazine, hippurylhydrazine, s-acetylbenzoylhydrazine, bidimethylazimethylene, isopropylideneacetylhydrazine, and isopropylidenebenzoylhydrazine. The introduction of such acyl radicals as acetyl, malonyl, ethylic carboxyl, hydroxybenzoyl, and hippuryl into the hydrazine molecule decreased the hypoglycemic action, while the introduction of the benzoyl radical increased this action slightly.

4. The introduction of a hydroxyl group into the m- or p-position in benzoylhydrazine decreased both toxicity and hypoglycemic potency, while an o-hydroxyl group decreased the hypoglycemic potency but increased the toxicity.

5. The introduction of thio groups, as thiocarbaminyl and dithioformyl, markedly increased the toxicity of hydrazine. The hypoglycemic effect was lost and a tendency toward the production of hyperglycemia was observed.

REFERENCES

- (1) UNDERHILL, F. P.: Jour. Biol. Chem., 1911, x, 159.
- (2) UNDERHILL, F. P., AND HOGAN, A. G.: Jour. Biol. Chem., 1915, xx, 203.
- (3) MACADAM, W.: Biochem. Jour., 1915, ix, 229.
- (4) UNDERHILL, F. P.: Jour. Biol. Chem., 1914, xvii, 295.
- (5) BODANSKY, M.: Jour. Biol. Chem., 1923, lviii, 799.
- (6) BODANSKY, M.: Jour. Pharmacol. and Exper. Therap., 1924, xxiii, 127.
- (7) GIBBS, W., AND HARE, H. A.: Amer. Chem. Jour., 1890, xii, 145.
- (8) GIBBS, W., AND HARE, H. A.: Amer. Chem. Jour., 1890, xii, 365.
- (9) KOLBE, H.: Jour. prakt. Chem., (ii), 1874, x, 89; xi, 9.
- (10) LEWIS, H. B.: Jour. Biol. Chem., 1913, xiv, 245.

